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13. ABSTRACT (Maximum 200 Words)
Metastatic prostate cancer is not a curable disease and approximately 30% of men undergoing radical prostectomy will relapse, so there is a need to identify new markers of development and progression of prostate cancer, particularly those that can be used as potential therapeutic targets. We are using aCGH and expression profiling to examine prostate cancers from men whom have been followed for 10 years on average. We are in the process of reviewing prostate specimens for cancer, macrodissecting them, extracting DNA and RNA for array based comparative genomic hybridization (aCGH) and expression profiling. In this progress report, we review our progress to date, which includes identifying many of the previous known genes found to be commonly amplified and deleted in prostate cancer, such as *TERT* and *HRAS*, and *NKX3-1* and *PTEN*, respectively. In addition, we have identified deletions and amplifications of novel genes not been shown to be changed in prostate cancer previously. Of greatest interest is GRB2, which functions upstream of the Ras signaling pathway. Ras signaling has been postulated to be important in androgen independent prostate cancer progression. In addition, targeted chemotherapy is being developed for GRB2 making it an important gene to understand further in prostate cancer.

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Introduction:
The purpose of the work is to use array based comparative genomic hybridization (aCGH) to finely map recurrent chromosomal amplifications and deletions in prostate cancer. Using aCGH, we will be able to characterize chromosomal changes in prostate cancer overall, and to compare samples from individuals that do not have recurrent disease to those that have undergone biochemical failure. For this project, we are studying prostate cancers that were removed and frozen for clinical purposes at least from 1992-1998 so that we have at least six years of follow-up on all cases. We are in the process of revewing prostate specimens for cancer, macrodissecting them, extracting DNA and RNA for array based comparative genomic hybridization (aCGH) and expression profiling. In this progress report, we review our progress to date, which includes identifying many of the previous known genes found to be commonly amplified and deleted in prostate cancer, such as *TERT* and *HRAS*, and *NKX3-1* and *PTEN*, respectively. We also have identified novel genes, in particular *GRB2*, which has not been shown to be amplified in prostate cancer previously. GRB2 functions upstream of the Ras signaling pathway, and Ras signaling has been postulated to be important in androgen independent prostate cancer progression. In addition, targeted chemotherapy is being developed for GRB2 making it an important gene to understand further in prostate cancer.

Body:
As the DOD IRB approval for the project was received in September, the project is at the 6 month point. Due to the delay, more than 25% of the monies received for the project are being are being carried over into the second year of the project to be used to further the research.

Specific Aim 1: To use aCGH to map regions of chromosomal amplification and deletion in primary prostate cancer specimens diagnosed under age 60
Specific Aim 2: To prioritize candidate genes within the three most consistently deleted or amplified chromosomal regions using expression array data from prostate cancers generated by us and mined from outside sources, so that we can identify specific genes involved in prostate cancer etiology and validate those genes
Specific Aim 3: To cluster aCGH data to detect an aCGH profile associated with biochemical failure after prostate cancer treatment

Pathological review of 137 patients (338 pieces of tissue) has been completed by Dr. Tomaszewski (Surgical Pathology). An additional 129 patients (278 pieces of tissue) remain to be examined for evidence of tumor. All specimens were deidentified as per the IRB protocol. We have identified thirty one patients with tumor, most of which have been macrodissected for DNA/RNA extraction. From 15 tumors, we extracted DNA and produced high quality aCGH data, which is discussed below. For the additional identified 16 tumors, DNA and RNA are in the process of being extracted and DNA placed onto the arrays. Our goal is to identify 80 tumors for the arrays. If we do not achieve our goal, we will either increase our age range of patients with prostate cancer or use achieved paraffin embedded tissue for which we have local IRB consent. We will consult with our Grants Officer prior to instituting either of these changes if necessary.
A database is in the process of being optimized for the pathological and clinical data specific to prostate cancer in Access with detailed clinical and pathological information and will be placed on a central server so that the pathologists and researchers will have access to it. Clinical data includes variable such as age of diagnosis, year of diagnosis, time to last follow up or recurrence of disease (based on rising PSA), PSA measurements before and after diagnosis (dates, method of measurement),

stage of disease, radiation or chemotherapy given, and family history of prostate cancer. Pathological data is captured for each block (matrix specific approach) and includes variables such as gleason grade, score, mostofi grade, % gland involvement, presences of prostate intraepithelial neoplasia (PIN), angiolymphatic invasion, margin postivity, capsular and seminal vesicle invasion, tumor volume and extracapsular extension. Each block is recorded as a separate instance with a common study identifier. The clinical data is linked to the pathological data through the use of the common identifier.

Preliminary analyses have been done of the most frequent amplifications and deletions in the sample set. We have not done a formal analysis as of yet, but the

Figure 1 – Frequent amplifications at 17q25.1 with GBR2 the most frequently amplified cancer gene highlighted – Red – deletion, Green - amplification

more advanced tumors (as defined by Gleason score) appear to have more genetic changes (as can be observed by eye in Figure 1). Overall, in our prelimina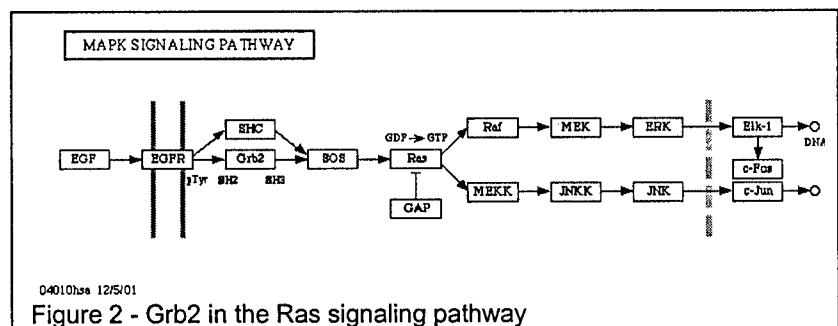ry data the most frequent amplification was seen on chromosome 17q25.1 (Figure 1), with other frequent amplifications (>50%) seen at 6p21.32, 7p22.1, 7q32.1, 7q36.3, 12q13.3, 16p11.2 and 20q13.3. Not unexpectedly many of these regions of amplification are at the telomeres and previously have been reported. The most frequently amplified cancer related genes are shown in Table 1. In our preliminary data, GRB2 is the most frequently amplified gene, and we have seen it

Name	Chrom	% Altered	Function
GRB2	17	60	growth factor signalling
COL11A2	6	53	collagen
SILV	12	53	melanin biosynthesis
FUS	16	53	fusion in malignant liposacroma
TERT	5	47	telomerase
MAD1L1	7	47	mitotic spindle-assembly checkpoint
HRAS	11	47	oncogene
GAS6	13	47	stimulates cell proliferation
BRCA1	17	47	breast cancer 1
PDGFA	7	47	platelet-derived growth factor A

Table 1 Most frequently amplified cancer related genes

commonly amplified in several other tumor types including breast and melanoma. The protein encoded by GRB2 binds the epidermal growth factor receptor (EGFR) and through SOS activates the RAS signaling pathway (see Figure 2, KEGG pathway). Previous literature has suggested that GRB2 is amplified and overexpressed in breast and bladder cancer cell lines[1, 2]. In addition, GRB2 directly interact with the Bcr portion of the Bcr-Abl fusion protein in chronic myelogenous leukemia (CML)[3]. Despite its importance in other cancer types, GRB2 previously has not been found to be amplified in prostate cancer. However, it has been hypothesized that activation of Ras signaling is sufficient and necessary
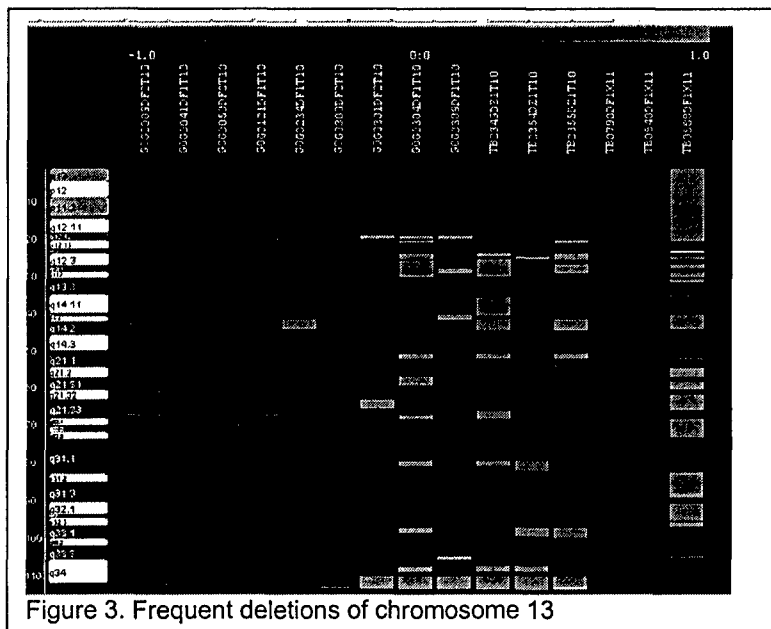

MAPK SIGNALING PATHWAY

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Figure 2 - Grb2 in the Ras signaling pathway

Figure 3. Frequent deletions of chromosome 13

for the progression of androgen independent prostate cancer[4]. While upregulation of GBR2 may not be enough to drive prostate cancer progression, it may act in concert with other receptor tyrosine kinases in prostate cancer. Other genes of interest that are amplified include *TERT* (telomerase), *HRAS* and *GAS6*. Telomerase has been previously shown to be amplified in 67-93% prostate cancers and is significantly associated with high grade prostate cancers[5]. HRAS also has been identified as amplified in hormone resistant tumors as compared to prior to hormone treatment (p=0.005)[6]. GAS6 in conjunction with Axl demonstrates a mitogenic effect and induces proliferation in prostate cancer cell lines[7]. In addition, amplification at 11q13.1 (specifically *MEN1*) has been seen to be associated with recurrence. We also see amplifications at 11q13.1 in 40% of our sample, but do not yet have the power to determine if this specific amplification is associated with recurrences. Our analyses are identifying genes that have been seen in progressive prostate cancer as the goal of our project.

In our preliminary data, the most frequently deleted regions (>50%) have been seen at 13q22.3-31.1, 1p13.2, 8p23.2 and 8p21.3. Deletions on chromosomes 13 and 8 are reported commonly in prostate cancer. We can specifically visualize the deletion on 8p in our CGH Browser, as shown in Figure 4. The most commonly deleted genes in our prostate cancer sample set include *NKX3-1* and *PTEN* on 8p21.1 and 10q23.21 in 60% of samples, both of which have been previously implicated as tumor suppressor gene important in prostate cancer. Deletions at 8p23.2 have been associated with advanced prostate cancer in multiple studies[8], specifically of *CSMD1*. We find deletions of *CSMD1* in 40% of our sample set, and as previously noted associated with more advanced prostate cancers. We have noted homozygous deletions at three locations: 1q24.1, 2p25.3 (telomere), and 4p15.33. Homozygous deletions can provide important clues about tumor suppressor gene locations, and we will be examining these more closely in the future. As with the

Figure 4 – Deletion at 8p11.2-23.2

Figure 5 – Common specific deletion of *PTEN*

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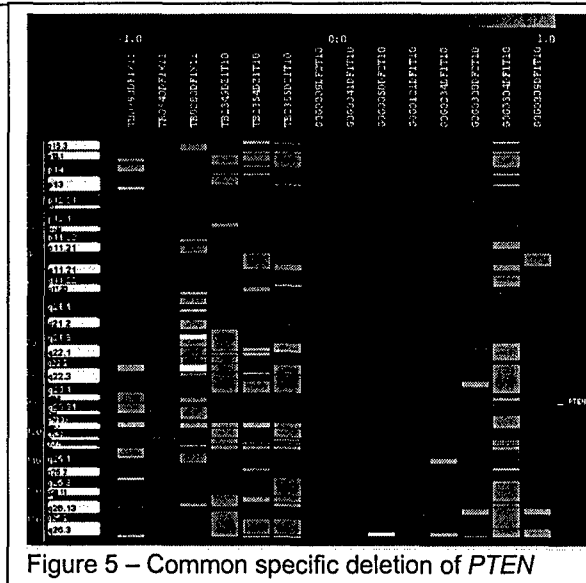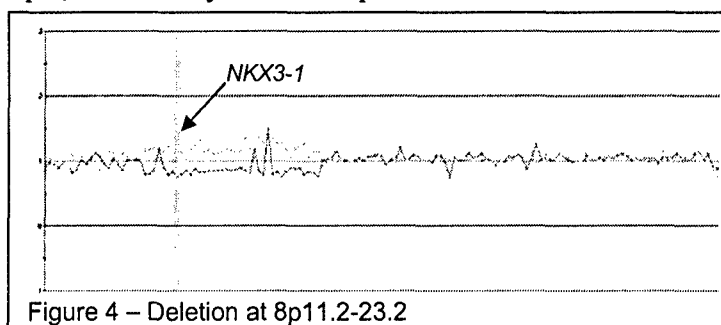
amplifications that we noted, many of the deletions we observe have been previously seen in prostate cancer validating our technique.

Among those tumors placed upon the arrays identified from the project, 50% of the tumors were from patients that experienced biochemical failure with a median time to recurrence of 39 months (range 20-64 months). The patients without biochemical failure had been followed for a minimum of 10 years. We are continuing to accumulate data to use for Specific Aim 3. Paris et al. have published a paper suggesting that gain at 11q13.1 predicts recurrence independent of stage and grade; they specifically examined the MEN1 gene as predictor of progression[8].

Key Research Accomplishments:
- Pathological review of prostate specimens from 137 patients (338 tissues)
- Optimization of a database specific to prostate cancer for pathological and clinical data for this study
- Producing aCGH data on 15 tumors, with DNA from 16 more tumors ready to be placed upon the arrays
- Validating our technique by identifying genes previously shown to be amplified (*TERT* and *HRAS*) and deleted (*PTEN* and *NKX3-1*) in progressive prostate cancer
- Identified novel genes amplified in prostate cancer, *GRB2* and *GAS6* for further evaluation. Both genes have potentially important roles in prostate cancer progression

Reportable Outcomes:
- We are in the process of optimizing a prostate cancer specific database for storage of clinical and pathological data.
- We have developed a tissue bank of prostate cancer samples that can be used for this and future research.

Conclusions:
The data that have been generated at this point are preliminary. Nonetheless, we are starting to identify novel genes that may be important in prostate cancer progression. In particular, GRB2 is of interest as it is phosphorylated by EGFR, acts upstream of the Ras signaling pathway and has been previously implicated as overexpressed in multiple cancer types. Thus, GRB2 has been identified as potential chemotherapeutic target as blocking the binding of Grb2 to the GDP-releasing protein SoS abrogates the activation of Ras. A macrocyclic tetrapeptide mimetic has been identified that binds to GRB2 and blocks transduction[9-11]. With this macrolide, anti-mitogenic effects against erbB-2-dependent breast cancers were achieved at non-cytotoxic concentrations. In addition, ZD1839 (Iressa) acts to block phosphorylation of GRB2 by EGFR[12]. Finally, high affinity agents against GRB2 are being developed as second line agents to be used when Gleevac fails for CML due to its interact with Bcr-Abl fusion protein[3]. If we validate that this gene is amplified and overexpressed in prostate cancer, these drugs could be potentially used for progressive prostate cancer.

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